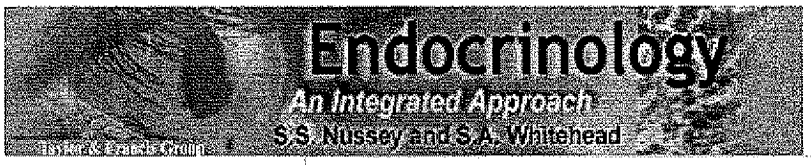
Exhibit A

Exhibit A



Box 6.14 Measurement of LH, FSH and hCG

Assay method	Advantages	Disadvantages
Radioimmunoassays (polyclonal Abs)	Cheap, sensitive, rapid	May not reflect biologica activity
IRMAs or ELISAs (monoclonal Abs (see Box 3.25)	Cheap, sensitive, rapid	May not reflect biologica activity
Receptor binding (see Box 3.15)	Measures hormone binding to receptors	Detects deglycosylated hormone that is inactive
Bioassay (see below)	Expensive, less sensitive and reproducible	Measures bioactive hormone

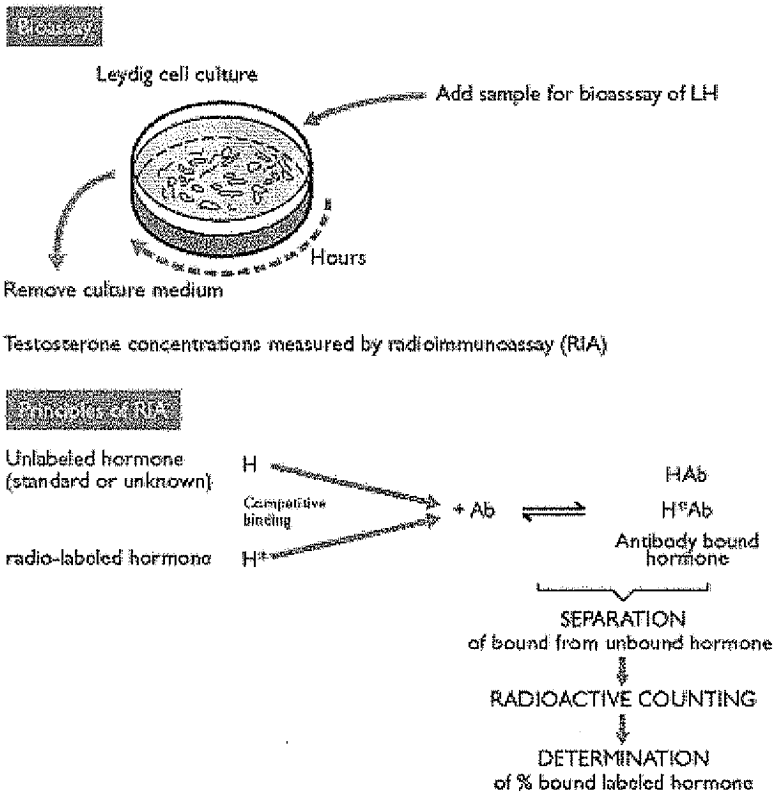


Exhibit B

GENEReviews
Funded by the NIH • Developed at the University of Washington, Seattle

PROP1- Related Combined Pituitary Hormone Deficiency (CPHD)

[*CPHD*]

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Initial Posting: December 7, 2000.
Last Update: November 21, 2005.

Summary

Disease characteristics. *PROP1*-related combined pituitary hormone deficiency (CPHD) is associated with deficiencies of growth hormone (GH); thyroid-stimulating hormone (TSH); the two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH); prolactin (PrL); and occasionally adrenocorticotrophic hormone (ACTH). Most affected individuals are ascertained because of growth failure and failure to thrive starting in infancy or early childhood (range: ~9 months to ~8 years). Hypothyroidism is usually mild and occurs in later infancy and childhood. Affected individuals can have absent or delayed and incomplete secondary sexual development with infertility. Males usually have a small penis and small testes. Some females experience menarche, but subsequently require hormone replacement therapy. ACTH deficiency is less common and, when present, usually occurs in adolescence or adulthood.

Diagnosis/testing. Testing for deficient secretion of GH, TSH, LH, FSH, PrL, and ACTH establishes the diagnosis of CPHD; *PROP1* is the only gene associated with *PROP1*-related CPHD. Targeted mutation analysis for the common recurring deletion in which three AG repeats are reduced to two

repeats is available clinically. This mutation accounts for 55% of familial cases of CPHD and 12% of nonfamilial cases of CPHD.

Management. GH deficiency is treated with injection of biosynthetic growth hormone from the time of diagnosis until approximately 17 years of age or longer. TSH deficiency is treated by thyroid hormone replacement in the form of L-thyroxine. Infants with micropenis are treated with testosterone. Hormone replacement to induce secondary sex characteristics can be initiated in males at 12 to 13 years with monthly injections of testosterone enanthate and in females at 11 to 12 years with conjugated estrogens or ethinyl estradiol and later by cycling with estrogen and progesterone. Children with untreated growth hormone deficiency receive sex hormone replacement in lower doses at a later age. Fertility in both sexes is possible with administration of gonadotropins. Management of ACTH deficiency is treated with oral hydrocortisone.

Genetic counseling. *PROP1*-related CPHD is inherited in an autosomal recessive manner. At conception, the sibs of an affected individual have a 25% chance of being affected, a 50% chance of being asymptomatic carriers, and a 25% chance of being unaffected and not carriers. Once an at-risk sib is known to be unaffected, the chance of his/her being a carrier is 2/3. Prenatal testing for pregnancies at 25% risk for *PROP1*-related CPHD is possible when both disease-causing *PROP1* mutations are known.

Diagnosis

Clinical Diagnosis

PROP1-related combined pituitary hormone deficiency (CPHD) is associated with deficiencies of growth hormone (GH); thyroid-stimulating hormone (TSH); the two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH); prolactin (PrL); and occasionally adrenocorticotrophic hormone (ACTH).

Affected individuals are usually ascertained because of short stature; diagnosis requires the presence of GH deficiency and at least one other pituitary hormone deficiency (Wu 1998) **and/or** identification of two *PROP1* mutations.

Growth hormone (GH) deficiency is suspected in children with the following features:

- Neonatal hypoglycemia

  and/or

- Proportionate short stature and delayed bone maturation (in the absence of an inherited bone dysplasia or chronic disease) with the following growth patterns [Rosenfeld 1996]:
  - Severe short stature with height more than three standard deviations (SD) below the mean for age

    or

  - Moderate short stature with height two to three SD below the mean for age and growth deceleration with height velocity less than 25th percentile for age

    or

  - Severe growth deceleration with height velocity less than 5th percentile for age

Thyroid-stimulating hormone (TSH) deficiency is suspected in children with growth failure, poor weight gain, and delayed bone maturation. Infants with congenital hypothyroidism rarely have signs in the first month of life. Signs and symptoms that may be apparent later are large posterior fontanelle (more than one centimeter in diameter), jaundice that lasts for more than one week after birth, macroglossia, hoarse cry, distended abdomen, umbilical hernia, and hypotonia.

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) deficiency is suspected in the following:

- Newborn males with micropenis (stretched penile length less than 2.5 cm in a term infant) without hypospadias with or without cryptorchidism
- Adolescent males with onset of puberty after age 14 years or cessation of secondary sexual development
- Adolescent females with lack of breast development or menses by age 14 years

Prolactin (PrL) deficiency is suspected in females with impaired lactation.

Adrenocorticotrophic hormone (ACTH) deficiency is suspected in children with persistent weakness, fever, abdominal pain, anorexia, and weight loss. Signs of acute ACTH deficiency include acute hypotension, dehydration, and shock accompanied by hyponatremia, hyperkalemia, and hypoglycemia.

Testing

Testing concomitantly for deficiency secretion of GH, TSH, LH, FSH, PrL, and ACTH should be done for diagnosis and management [Phillips 1995, Rimoin & Phillips 1997].

Deficiencies of all pituitary hormones may be assessed simultaneously using the triple test (exogenous GnRH [gonadotropin-releasing hormone], TRH [thyrotropin-releasing hormone], and insulin-induced hypoglycemia). GnRH should increase FSH and LH. TRH should increase TSH and PrL. Hypoglycemia (with a blood sugar less than 40 mg/dL or half the baseline value) should result in an increase in the stress hormones prolactin, growth hormone, and cortisol.

Growth hormone (GH) deficiency. Even in the appropriate clinical setting, the diagnosis of GH deficiency remains problematic because of the difficulty in measuring physiologic GH secretion. Provocative tests of GH secretion are widely used in the diagnosis of GH deficiency, although they are associated with a high false-positive rate. Stimuli include exercise, arginine, L-Dopa, clonidine, insulin, insulin-arginine, glucagon, and propranolol. A peak serum concentration of GH greater than 7-10 ng/mL on one test rules out the diagnosis of GH deficiency.

- Confirmatory study: serum concentration of GH less than 7-10 ng/dL on two provocative tests

Thyroid-stimulating hormone (TSH) deficiency

- Low serum T_4 concentration 1.0 µg/dL below normal for age with a low serum TSH concentration (normal: 0.1 mU/L to 4.5-5.5 mU/L)
- Confirmatory study: subnormal increase in serum concentration of TSH 30 minutes after infusion of TRH

Note: All newborn screening programs for congenital hypothyroidism screen for elevated TSH; only those programs that also use initial T_4 measurements detect infants with low

TSH.

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) deficiency

- Low serum concentrations of LH, FSH, and low serum testosterone concentration in males or low serum estradiol concentration [and/or the lack of a progestin-induced withdrawal bleeding] in females 14 years of age or older
- Confirmatory study: subnormal increase in serum concentration of LH and FSH following infusion of GnRH in an individual 14 years of age or older

Note: No absolute cutoff values have been established.

Prolactin (PrL) deficiency

- Very low or undetectable baseline prolactin
- Confirmatory study: absent response to TRH stimulation

Adrenocorticotrophic hormone (ACTH) deficiency

- Low serum concentration of sodium and glucose and elevated serum concentration of potassium in an acutely ill individual
- Serum ACTH concentration that is inappropriately low in the face of a low serum concentration of cortisol (Note: The blood for the ACTH assay needs to be collected properly on ice and sent to the laboratory expeditiously.)
- Normal renin-aldosterone axis
- Confirmatory study: subnormal increase in serum ACTH concentration in response to CRH (corticotropin releasing hormone) suggests a pituitary etiology of ACTH deficiency. Insulin-induced hypoglycemia may also be used, but a subnormal response could indicate a hypothalamic or a pituitary cause.

Molecular Genetic Testing

GeneReviews designates a molecular genetic test as clinically available only if the test is listed in the GeneTests Laboratory Directory *by either a US CLIA-licensed laboratory or a non-US clinical laboratory. GeneTests does not verify laboratory-submitted information or warrant any aspect of a laboratory's licensure or performance. Clinicians must communicate directly with the laboratories to verify information.*—ED.

Gene. *PROP1* is the only gene associated with *PROP1*-related CPHD.

Molecular genetic testing: Clinical uses

- Confirmatory diagnostic testing
- Carrier testing
- Prenatal diagnosis

Molecular genetic testing: Clinical method

Targeted mutation analysis. The common recurring deletion in which three AG repeats are reduced to two repeats (301-302delAG) accounts for 55% of alleles in familial cases and 12% of alleles in simplex cases (i.e., single occurrence in a family) of CPHD.

Sequence analysis. The mutation detection rate varies by study suggesting either bias in ascertainment in some studies or variation in the frequency of PROPI mutations between populations of different ethnic origins.

- In several international centers (UK, India, and Poland), *PROP1* mutations were identified in only 1-2% of individuals representing simplex cases of CPHD and almost 30% of familial cases [Turton et al 2005].
- No *PROP1* mutations were identified in ten simplex cases and 12 familial cases of CPHD in the UK [Rainbow et al 2005].
- No *PROP1* mutations were identified in 32 unrelated CPHD probands from Austrailia [McLennan et al 2003] or 27 children with CPHD from 26 families from the West Midlands, UK [Rainbow et al 2005].

Table 1 summarizes molecular genetic testing for this disorder.

Table 1. Molecular Genetic Testing of *PROP1*-Related Combined Pituitary Hormone Deficiency

Test Method	Mutations Detected	Mutation Detection Rate [1]	Test Availability
Targeted mutation analysis	301-302delAG	Familial 55% [2] Simplex 0-12% [3]	Clinical
Sequence analysis	*PROP1* sequence alterations	~0-25%	

1. These rates indicate ranges
2. Familial: More than one affected family member
3. Simplex: Single occurrence in a family

Interpretation of test results. For issues to consider in interpretation of sequence analysis results, click **here**.

Genetically Related Disorders

No other phenotypes are associated with mutations in *PROP1.*

Note: There is no evidence that individuals with isolated deficiency of gonadotropins (hypogonadotropic hypogonadism) have mutations in *PROP1* [Park et al 2004], although this might be presumed to be a milder phenotypic variant than full CPHD.

Clinical Description

Natural History

PROP1 gene mutations are associated with deficiencies of growth hormone (GH), thyroid-stimulating hormone (TSH), gonadotropins (FSH and LH), prolactin (PrL), and adrenocorticotrophic hormone (ACTH). The secretion of all these pituitary-derived hormones declines gradually with age; often the order of appearance of hormone deficiency is GH, LH and FSH, TSH, and ACTH. The degree of hormone deficiency and the age of onset of the deficiency are variable even within the same family. In a follow

up study of nine individuals with *PROP1* mutations, all seven who had reached the age of puberty required steroid hormone replacement therapy. Repeated testing of pituitary function indicated a decline over time; all individuals developed some degree of adrenal insufficiency [Bottner et al 2004].

Most affected children have normal birth weight and birth length and an uncomplicated perinatal period. Growth failure and failure to thrive begin in infancy or early childhood (range: ~9 months to ~8 years). Rarely, hypothyroidism is the presenting finding [Fluck et al 1998].

Individuals with *PROP1*-related CPHD who have untreated growth hormone deficiency have proportional short stature (i.e., less than four centimeters difference between length of arm span and height) with proportionately small hands and feet. Height is usually profoundly reduced, with SDS scores of more than -3.7 [Bottner et al 2004; Reynaud, Chadli-Chaieb et al 2004].

Affected individuals can have absent or delayed and incomplete secondary sexual development with infertility.

- Males usually have a small penis and small testes.
- Some females experience menarche before requiring hormone replacement therapy [Fluck et al 1998].

Severe deficiency of GH and insulin-like growth factor 1 (IGF-1) with mild hypothyroidism and absence of secondary sexual development result in significant growth failure. In one Brazilian family with eight affected individuals, adult heights ranged from -5.9 to -9.6 SD below the mean.

Hypothyroidism is usually mild and occurs in later infancy and childhood. Since it is usually not congenital or severe, it is not associated with mental deficiency.

It was initially thought that ACTH deficiency was uncommon and, when present, usually occurred in adolescence or adulthood. However, longer follow-up has shown that some degree of adrenal failure may occur in most individuals with *PROP1* mutations [Bottner et al 2004].

An 8° to 20° limitation of extension of the elbows that increases with age has been observed. Facies are characterized as "immature," with a depressed nasal bridge and relative decrease in the vertical dimensions of the face.

Obesity is uncommon in childhood and is more common in adulthood.

Hypoglycemia is not usually reported. Intelligence is normal.

On imaging studies, the pituitary may initially appear diffusely enlarged in childhood and then reduced in size in adolescence or adulthood [Mendonca et al 1999; Riepe et al 2001; Reynaud, Chadli-Chaieb et al 2004; Tatsumi et al 2004; Voutetakis, Argyropoulou et al 2004; Voutetakis, Maniati-Christidi et al 2004].

The sella turcica may be normal in size or enlarged, or may appear "empty."

Genotype-Phenotype Correlations

No genotype-phenotype correlations exist.

Penetrance

Exhibit C

Exhibit C

1: J Soc Gynecol Investig. 2004 Sep;11(6):393-8.

Dependence of 3',5'-cyclic adenosine monophosphate--stimulated gonadotropin-releasing hormone release on intracellular calcium levels and L-type calcium channels in superfused GT1-7 neurons.

Chen EC, **Javors MA**, **Norris C**, **Siler-Khodr T**, **Schenken RS**, **King TS**.

Department of Obstetrics and Gynecology, Southern California Permanente Medical Group, Los Angeles, California, USA.

OBJECTIVE: Immortalized GT1-7 neurons were used to characterize the interactive roles of adenylate cyclase-3',5'-cyclic adenosine monophosphate (cAMP) and L-type calcium channels on gonadotropin-releasing hormone (GnRH) release. METHODS: Dibutyryl (db)-cAMP was used as an active analog of endogenous cAMP, and forskolin was used to activate adenylate cyclase. Extracellular calcium was chelated using EGTA and L-type calcium channels were blocked using nimodipine. The selective Ca2+ ionophore A23187 was employed to increase intracellular calcium levels. GT1-7 neurons were grown on Cytodex-3 beads (Pharmacia Biotech, Uppsala, Sweden) and placed in special superfusion microchambers. The cells were superfused at a rate of 6.2 mL/h with media 199 (M-199; Gibco, Grand Island, NY; pH 7.35, 37C); effluent fractions were collected at 5-minute intervals for analysis of GnRH concentrations by radioimmunoassay. RESULTS: Basal GnRH release from superfused GT1-7 neurons ranged from 10 to 62 pg. min(-1). mL(-1). Coexposure of the cells to forskolin and A23187 produced an additive effect on stimulated release of GnRH. Cells exposed to 1 microM of forskolin (an activator of adenylate cyclase) for 5 minutes showed a 2.6-fold increase in GnRH release. Likewise, the addition of 100 microM of db-cAMP to the superfusion for 5 minutes demonstrated a 2.3-fold increase in the amplitude of GnRH secretion. Maintaining the superfused cells in medium containing 5 mM EGTA had no obvious effect on basal GnRH release but blocked the effect of db-cAMP to increase GnRH release. Similarly, the

addition of 10 microM nimodipine to the superfusion medium blocked db-cAMP-stimulated GnRH release. CONCLUSIONS: These findings provide additional evidence that cAMP-mediated GnRH release from GT1-7 neurons is dependent on influx of extracellular calcium via L-type Ca2+ channels.

PMID: 15350253 [PubMed - indexed for MEDLINE]

Patient Drug Information

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Exhibit D

Exhibit D

Kits

for quantitative determination of rat, mouse and human CRF

Corticotropin releasing factor/CRF ELISA Kit and CRF Related products

Back Ground

Corticotropin releasing factor (CRF, also CRH) was initially isolated from ovine hypothalamus by Vale et al., in 1981, and identified as a novel neuropeptide comprising 41 amino acid residues with molecular weight 4758 [1]. Later human CRF[2] and rat CRF[3] were also isolated and identified [4]. CRF in anterior pituitary promotes the synthesis and secretion of ACTH, a main factor of hypothalamus-pituitary-adrenal (HPA) axis. In the rat and human, CRF distributes mainly in hypothalamus, but it was also found in spinal cord, stomach, spleen, duodenum, adrenal and placenta. In addition, immunochemical evidence supported the wide distribution of the peptide throughout the central nervous system (CNS) such as olfactory bulb, retina and central auditory system in the rat. However because of the wide distribution, it is still disputing about CRF whether its blood level can reflect only the function of HPA axis [5].

The relationships between CRF and stress, CRF and Alzheimer disease (AD) were attracted much attention recently. In fact the peptide was also suggested to regulate endocrine, autonomic and behavioral responses to stress, based on an experiment with acute and chronic stress rat models that showed endocrine function changes similar to those seen in patients with depression [5]. CRF in serial cerebrospinal fluid(CSF)of patients with depression was strikingly reduced as compared to those of normal subjects [7] , [8]. The mean CRF and ACTH levels in the CSF of AD patients were significantly lower than those of healthy controls [9]. Only in the cortices of those with mild dementia, CRF was reduced significantly, thus CRF was proposed to serve as a potential neurochemical marker of early dementia and possible early AD [10].

A large proportion of the CRF in human brain was shown to be in the form of complex with its binding protein (CRF-BP). CRF molecule in the complex is unavailable for activation of the CRF receptor. Accordingly reductions in total CRF do not necessarily predict reductions of bioactive free CRF, and the levels of total CRF and CRF in the form of complex (CRF/CRF-BP) were suggested to be the main factors determining the quantity of bioactive free CRF in human brain [11]. In AD there have been observed dramatic reductions in the content of free CRF in the brain and thus displacement of CRF from CRF-BP was proposed as a possible treatment for AD [12]. In primary neuron culture, CRF exhibited protective effect against cell death induced by amyloid-beta peptide, suggesting that disturbances in HPA axis function can occur independently of alteration in CRF mRNA levels in AD brain and further suggesting an additional role for CRF in protecting neurons against cell death [13]. On the other hand, Yanaihara et al., demonstrated immunoreative CRF in various neuroendocrine tumors, and suggested that the blood level of the peptide might be used as a tumor marker [6].

All these information urge crucial importance of the measurement of CRF in the brain especially of experimental animals not only for analysis of the function of CRF

now.

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in CNS, but also for research in the fields of stress response and AD

Reference

1) Vale, W., Spiess J., Rivier C. and Rivier, J. (1981): Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science., 213: 1394 - 1397.

2) Shibuhara, S., Morimoto, Y., Furutani, Y., Notake, M., Takahashi, H., Shimizu, S., Horikawa, S. and Numa, S. (1983): Isolation and sequence analysis of the human corticotropin-releasing factor precusor gene. EMBO. J. 2: 775 – 779.

3) Rivier, J., Spiess J. and Vale, W. (1983): Characterization of rat hypothalamic corticotropin-releasing factor., 80: 4851 - 4855.

4) Vaughan, J., Donaldson, C., Bittencourt, J., Perrin MH., Lewis, K., Sutton, S., Chan, R., Turnbull, AV., Lovejoy, D. and Rivier, C. et al. (1974): Urocortin, a mammalian neuropeptide related to fish urocortin I and to corticotropin-releasing factor. Nature, 378: 287 - 292.

5) Chappell, PB., Smith, MA., Kilts, CD., Bissette, G., Ritchie, J., Anderson, C. and Nemeroff CB. (1986): Alterations in corticotropin-releasing factor-like immunoreactivity in discrete rat brain regions after acute and chronic stress. J Neurosci. 6: 2908-2914.

6) Tsuchihashi, T., Yamaguchi, K., Abe, K., Yanaihara, N. and Saito S. (1992): Production of immunoreactive corticotropin-releasing hormone in various neuroendocrine tumors. Jpn J Clin Oncol. 22: 232-237.

7) Ur, E. and Grossman, A. (1992) Corticotropin-releasing hormone in health and disease: an update. Acta Endocrinol (Copenh). 127: 193-199. Review.

8) Geracioti, TD. Jr., Orth, DN., Ekhator, NN., Blumenkopf, B. and Loosen, PT. (1992) Serial cerebrospinal fluid corticotropin-releasing hormone concentrations in healthy and depressed humans. J Clin Endocrinol Metab. 74: 1325-1330.

9) May, C., Rapoport, SI., Tomai, TP., Chrousos, GP and Gold, PW. (1987) Cerebrospinal fluid concentrations of corticotropin-releasing hormone (CRH) and corticotropin (ACTH) are reduced in patients with Alzheimer's disease. Neurology. 37: 535-538.

10) Davis, KL., Mohs, RC., Marin, DB., Purohit, DP., Perl, DP., Lantz, M., Austin, G. and Haroutunian, V. (1999) Neuropeptide abnormalities in patients with early Alzheimer disease. Arch Gen Psychiatry. 56, 981-987.

11) Behan, DP., Khongsaly, O., Owens, MJ., Chung, HD., Nemeroff, CB. and De Souza, EB. (1997) Corticotropin-releasing factor (CRF), CRF-binding protein (CRF-BP), and CRF/CRF-BP complex in Alzheimer's disease and control postmortem human brain. J Neurochem. 68: 2053-2060.

12) Behan, DP., Heinrichs, SC., Troncoso, JC., Liu, XJ., Kawas, CH., Ling, N. and De Souz, EB. (1995) Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. Nature 378: 284-287.

13) Pedersen, WA., McCullers, D., Culmsee, C., Haughey, NJ., Herman, JP. and Mattson, MP. (2001) Corticotropin-releasing hormone protects neurons against insults relevant to the pathogenesis of Alzheimer's disease. Neurobiol Dis. 8: 492-503.

Features

- **Specificity**
  The ELISA kit shows following each cross reactivity of rat, mouse and human peptides.

Peptide	Cross reactivity	Peptide	Cross reactivity
Rat/Human CRF(1-14)	100%	Mouse Urocortin 2	0%
Rat/Human CRF(17-41)	0.10%	Mouse Urocortin 3	0%
Human ACTH	0.01%	PACAP27	0%
Rat ACTH	0.01%	PACAP38	0%
Human Urocortin	0.01%	Human/Porcine VIP	0%

Rat/Mouse Urocortin	0.01%		

Component

		Form	Quantity	Main Ingredient
1.	Antibody coated plate	MTP*1	1 plate(96 wells)	Rabbit anti rat/mouse/human CRF antibody
2.	CRF standard	lyophilized	1 vial (20ng)	Synthetic rat/mouse/human CRF(1-14)
3.	Labeled antibody solution	liquid	1 vial	Biotinylated anti rat/human CRF antibody
4.	SA-HRP solution	liquid	1 bottle (12 mL)	HRP labeled streptoavidin
5.	Buffer solution	liquid	1 bottle (20 mL)	Phosphate buffer
6.	Substrate buffer	liquid	1 bottle (26 mL)	0.015% Hydrogen Peroxide
7.	OPD tablet	tablet	2 tablets	o-Phenylenediamine hydrochloride
8.	Stopping solution	liquid	1 bottle (12 mL)	1M-H2SO4
9.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
10.	Adhesive foil		3 sheets	

MTP*1 .... . Microtiration plate

product list

	Product Name	Cat#	Assay range	Assay sample volume	Assay time	Quantity	Price
	CRF, ELISA Data Sheet	YII-YK130-EX	0.313~20 ng/mL	50 µL (Brain Tissue extract)	6.5 hr.	1 KIT	N/A

Add to Cart Reset

CRF ELISA Kit [HIGH SENSITIVITY]

	Product Name	Cat#	Assay range	Assay sample volume	Assay time	Quantity	Price
☐	CRF, ELISA (High Sensitivity) Data Sheet	YII-YK131-EX	0.078~2.5 ng/mL	50µL (Plasma, Brain Tissue Extract)	7.5hr.	1 KIT	**¥79,000** $753 €608

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Related Products[antibody]

	Product Name	Cat#	Antigen	Host	Quantity	Price

☐	Anti CRF (24-41) Data Sheet	YII-Y210-EX	(human, mouse, rat), carrier free	RAB	50 UL	**¥35,000** $334 €270
☐	Anti CRF (3-41) Data Sheet	YII-Y211-EX	(human, mouse, rat)-pTG conjugate	RAB	50 UL	**¥35,000** $334 €270

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Exhibit E

Exhibit E

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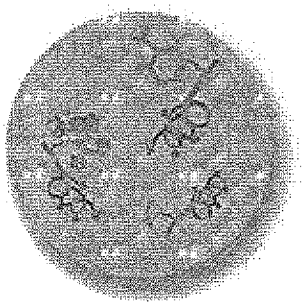
Products—Nuclear Hormone Receptors

HitHunter™ Cortisol Plus Assay

Detection: EFC Chemiluminescent Detection

Description | **Benefits** | **Assay Principle** | **Product Details** | **Performance**



Overview & Description

Cortisol is a steroid hormone that is produced and secreted by adrenal cortex. As the major glucocorticoid in the human body, it is involved in stress adaptation, blood pressure elevation and Na+ uptake, and has numerous effects on the immune system.

The HitHunter™ Cortisol Plus Assay Kit is an assay suitable for the measurement of cortisol in a competitive immunoassay format using EFC detection.

Product	Part No.	Size (384-well)
HitHunter™ Cortisol Plus Assay	90-0089	800 tests
	90-0089 L	10,000 tests

L

Request a quote, Click Here

Features and Benefits

» **Directly measure cortisol or cortisol expressed from microsomes**
» **Flexible**
  •Compatible with cells or purified enzyme preparations
» **Specific Antibody**
  •Does not bind to other molecules in the cortisol pathway
» **Reduced Interference**
  •Chemiluminescent detection reduces interference from fluorescent compounds, lowering false positives
» **Robust**
  •Large signal to background ratios for superior signal differentiation and assay performance

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Related Documents

» HitHunter Cortisol Assay Principle
» HitHunter Cortisol Assay Protocol
» SBS 04 - Wyeth Cortisol Screen

Related Links

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» Google Search

Measuring cortisol using HitHunter EFC Detection

HitHunter Cortisol Plus Assay is a rapid, homogeneous method for measuring cortisol using Enzyme Fragment Complementation (EFC) as the detection method. Free cortisol competes for antibody binding against labeled ED-cortisol conjugate, a small peptide fragment of ß-galactosidase (ß-gal). Unbound ED-cortisol is free to complement with EA, an inactive ß-gal EFC enzyme, to form active enzyme, which subsequently hydrolyzes luminescent substrate for EFC detection by a microplate reader or CCD camera. The amount of signal generated is proportional to the amount of cortisol that is unbound by antibody.

view a graphic representation

Product details

Kit Contents

» ED-cortisol conjugate, EFC Label
» Cortisol Antibody
» EFC detection reagents
» Cortisol Standard

Instrument Requirements

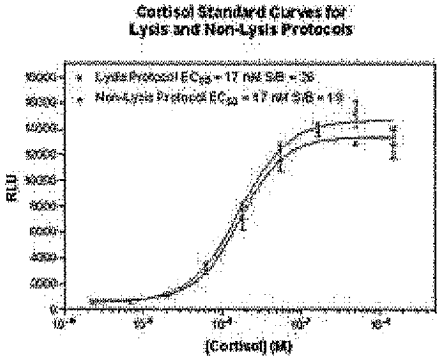
» Luminometer, Multimode Microplate Reader or CCD Camera

Flexible Assay Characteristics
HitHunter™ Cortisol Assay is a reliable and simple method for the detection of cortisol. It is designed to be extremely flexible for a variety of formats suitable for discovery research for both therapeutic and HTS programs.

Easily Automated	Homogeneous protocols that are easily automated on existing liquid handlers
Scaleable	Adpatable protocols for 96-, 384-, and 1536-well applications
Stable Signal	Measurements can be made up to 24 hours

back to top

Performance: Standard Curve
A typical standard curve is shown in the graph. Hithunter Cortisol has been designed to exhibit robust and reliable assay performance as defined by the following parameters:
EC50: <20 nM
Z' factor > 0.75



Cortisol standard was serially diluted with PBS Buffer and assayed in the Cortisol assay. The assay can measure a molar concentration range 1.5 x 10-6 to 2.29 x 10-10 [M]. The readout illustrates a positive reading from low to high levels of cortisol. The signal is stable up to 24 hours.

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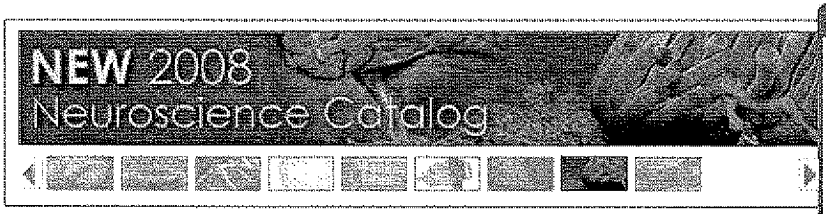
Exhibit F

Exhibit F



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All contact info

582701 Testosterone EIA Kit

Testosterone ELISA Kit

• See below for pricing and ordering
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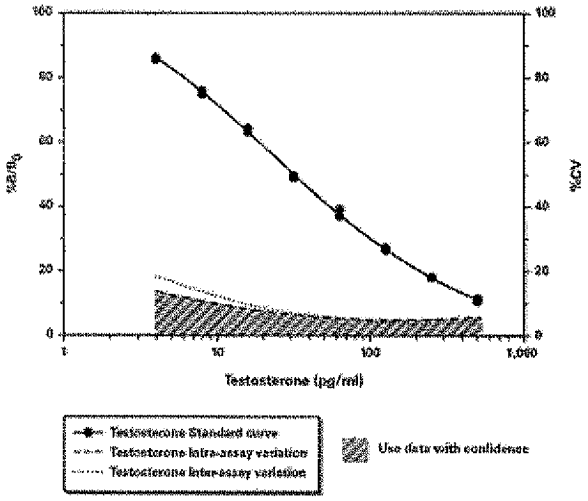

You may be eligible to receive a **free sample** Testosterone EIA Kit under the Cayman Challenge program.

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Data | Description | Articles | Related Products | Kit Contents

Sensitivity: 50% B/B_0: 32 pg/ml; **80% B/B_0:** 6 pg/ml · Testosterone is the prototypic and predominant circulating androgenic steroid. It plays a major role in the growth and function of many reproductive and non-reproductive tissues and organs including muscle, liver, and brain, directing the development of the male phenotype during embryogenesis and at puberty. Testosterone is synthesized from 17α-hydroxy progesterone by the enzymes 17,20-lyase and 17β-hydroxysteroid dehydrogenase in the gonads of both males and females. In many target cells it is reduced to 5α-dihydro testosterone, which mediates many of the biological actions

of testosterone. It is then further metabolized to 17β-estradiol by aromatase.[1] Serum levels of testosterone range from 0.5 ng/ml in women to approximately 6-10 ng/ml in men, declining with age.[2]

1 Vance, D.E. Cholesterol and related derivatives. 2nd ed., 725-748 (1988).

2 Miller, W.L., Tyrrell, J.B. The adrenal cortex. 3rd edition, 555-711 (1995).

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